

EFFECTS OF WEATHERING ON THE TOXICITY OF THREE OFFSHORE AUSTRALIAN CRUDE OILS AND A DIESEL FUEL TO MARINE ANIMALS

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Abstract The evaporative weathering properties, chemical composition, and toxicity of three Australian Northwest Shelf crude oils and an Australian diesel fuel were evaluated. The crude oils include one each of a condensate, a light, and a medium crude oil. Between 23 and 100% of the mass of the oils is lost during evaporative weathering equivalent to about 1 week on the sea surface. During weathering, the oils lose most of their monocyclic aromatic hydrocarbons (MAHs) and phenols; concentrations increase of less volatile phenols and polycyclic aromatic hydrocarbons (PAHs). The acute toxicity of water-accommodated fractions (WAFs) of the fresh and weathered oils to six species of temperate and tropical marine animals ranges from 100% to about 11% WAF. The MAHs are the most important contributors to the acute toxicity of the WAFs of the fresh oils. The contribution of PAHs to WAF toxicity increases with weathering. About 58% of the hazard indices (HI: exposure concentration/acute toxic concentration) for the WAFs of the two light oils weathered for the equivalent of 1 d are attributable to PAHs. The toxicity of the WAFs of the condensate and light crude oil can be accounted for by MAHs, PAHs, and phenols; WAFs of the middle-weight crude oil and diesel fuel are higher than predicted based on their concentrations of total MAHs, PAHs, and phenols, indicating that other components of the WAFs are contributing to their toxicity. These components may include the unresolved complex mixture and polar compounds (resins).

Keywords Polycyclic aromatic hydrocarbons Monocyclic aromatic hydrocarbons Phenols

INTRODUCTION

The greatest potential for future discoveries of commercial quantities of oil and gas in Australia is on the continental shelf. The total undeveloped reserves of crude oil in the 12 million square kilometer area of the Australian Exclusive Economic Zone and Legal Continental Shelf is conservatively estimated to be about 20 billion barrels [1]. An area showing especially good potential is the continental shelf off northwestern Australia, an area of shallow tropical seas, with numerous small islands fringed with coral reefs and mangrove forests, and beaches that are important habitat for sea turtles, dugongs, many species of marine birds, and many other marine animals of intrinsic or commercial value [2]. It is the site of extensive offshore exploration for and development of oil and gas resources. Apache Energy (Perth, Australia) and its joint venture partners currently are developing oil and gas fields north of Barrow Island near the Lowendal and Montebello Islands (off the northwest coast of Western Australia). Because there are concerns about the possible adverse effects of accidental releases of oil on the tropical ecosystems of the Northwest Shelf, particularly coral reefs [1-3], Apache has undertaken studies of the composition, weathering behavior, and toxicity of crude oils produced by Apache on the Northwest Shelf. These studies will enable Apache to develop strategies for effective spill response should an accidental release occur [3].

Crude and refined oils from different reservoirs vary widely in chemical composition and physical properties [4]. These chemical and physical properties affect the fate of the oil following release to the ocean, the types of spill response methods

that might be effective for cleaning up the oil, and the effects of the oil on marine organisms and ecosystems.

Following a spill of crude or refined oil, several physical, chemical, and biological processes, collectively called weathering, interact to change the physical and chemical properties of the oil and thereby influence its effects on marine ecosystems and the effectiveness of various spill countermeasures for removing the oil from the environment [4]. The most important weathering processes include spreading, evaporation, dissolution, dispersion into the water column, formation of water-in-oil emulsions, photochemical oxidation, microbial degradation, adsorption to suspended particulate matter, and stranding on the shore or sedimentation to the seafloor.

The timing and magnitude of each weathering process is different following an oil spill. During the first few days after a release of crude oil to the ocean, the weathering processes most affecting the chemical and physical properties of the oil are spreading and evaporation [4]. For oils of all densities, about 50 to 70% of the amount of oil that ultimately will be lost by evaporation is lost within the first 10 to 12 h after a release [5]. Because of the importance of evaporation in the early weathering of oil on the sea surface, particularly in a tropical climate, the simulated weathering evaluated in this study is just evaporative weathering; other weathering processes were not evaluated empirically.

Because the compositions of crude and refined oils vary widely, changes in the compositions of the oils during evaporative weathering and the toxicities of the weathered fractions also vary widely. This study is the first attempt to relate the changes in physical and chemical properties of crude oils during evaporative weathering to changes in their chemical compositions and toxicities to marine animals.

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The objective of this study was to gain a better understanding of the potential consequences of oil spills for sensitive marine organisms and ecosystems on the Northwest Shelf of Australia through an analysis of the evaporative weathering and emulsion-forming properties of three representative crude oils and a diesel fuel used on offshore platforms on the Northwest Shelf of Australia and how weathering affects the chemical composition and toxicity of the oils. The focus of this paper is on the results of toxicity tests with the four oils and their weathered fractions. Concentrations of alkanes, monocyclic aromatic hydrocarbons (MAHs), polycyclic aromatic hydrocarbons (PAHs), selected sulfur heterocyclic compounds, and phenols were measured in the fresh and weathered oils and their water-accommodated fractions (WAFs). These are the compounds that make up a majority of the components of petroleum that can be resolved by conventional gas chromatographic methods and are thought to be the most important contributors to the toxicity of oil to marine animals. Detailed compositional information for the water-accommodated fractions of the four oils provides the information needed to identify the relative contributions of different organic chemicals to the marine toxicity of fresh and weathered crude and refined oils.

MATERIALS AND METHODS

Crude and refined oils

Approximately 20 L each of three crude oils and an Australian Standard 3570 diesel fuel were obtained from Apache from its exploration and production facilities on the Northwest Shelf of Australia. The crude oils include Campbell condensate, Wonnich crude oil, and Agincourt crude oil. A condensate is the crude oil that condenses from the natural gas production stream when it is brought to surface temperature and pressure. The diesel fuel is the type used to fuel machinery on production platforms on the Northwest Shelf. The four oils were stored in the cold and dark until the investigation was initiated.

Topping of crude oils

Each oil was artificially weathered by distillation (topping) under controlled conditions in the laboratory to simulate the evaporative weathering that might occur in crude oil released to surface waters of the Northwest Shelf [6,7]. The topping consisted of heating replicate samples of approximately 1,500 g of fresh oil to a predetermined temperature under an inert, flowing blanket of nitrogen gas in a standard distillation apparatus.

The liquid- and vapor-phase temperatures were monitored continuously during topping. The topping was halted when the vapor phase reached the desired topping temperature. Vapor-phase distillation temperatures of 150, 200, and 250°C were used to simulate on-the-water weathering equivalent to 1 to 3 h, 0.5 to 1 d, and 2 to 5 d, respectively, at 13°C air and water temperature with moderate wind conditions [6]. Estimates of weathering times on these sea surfaces were derived by comparing hydrocarbon compositions and concentrations in crude oils experimentally weathered in an open system at 13°C with hydrocarbon compositions and concentrations in the topped oil fractions. Simulated weathering times would be slightly shorter at higher air and water temperatures typical of the Northwest Shelf. For example, evaporative weathering for 24 h of a light crude oil would be about 27% at 13°C and 33% at 30°C.

The weight of the undistilled oil residue remaining and the

total volume of the distillate were measured and recorded at each topping temperature. The undistilled oil remaining after each distillation represents the topped or weathered oil fraction. The topped oil fractions, after cooling, were stored under nitrogen gas in sealed amber bottles at room temperature.

Analysis of aliphatic and total petroleum hydrocarbons

The fresh and weathered oils and their WAFs were analyzed for C₁₀ through C₃₆ *n*-alkanes, selected branched alkanes, and total resolved plus unresolved petroleum hydrocarbons (total petroleum hydrocarbons) (Appendix) according to modifications of U.S. Environmental Protection Agency (U.S. EPA) Methods 3510 and 3611 [8,9]. The total gas chromatographic hydrocarbon signature of fresh and topped residues of the oils was determined with a Hewlett-Packard 5890 Series II capillary gas chromatograph with flame ionization detection (Avondale, PA, USA). A 3- μ l aliquot of a 5 mg/ml dichloromethane dilution of each oil was injected, using splitless techniques, onto a 30-m DB-5 capillary column (J&W fused silica DB-5, 30-m, 0.32-mm internal diameter [i.d.], and 0.25- μ m film thickness; J&W Scientific, Folsom, CA, USA). The gas chromatograph oven was programmed from 35 to 320°C at 6°C/min. Prior to sample analysis, a six-point calibration curve containing *n*-C₁₀ through *n*-C₃₆ *n*-alkanes, pristane, and phytane was generated to demonstrate instrument calibration and performance.

Alkane and total hydrocarbon concentrations were quantified by the method of internal standards using 5-androstane. Total petroleum hydrocarbons (resolved plus unresolved) were quantified by the method of internal standards using the baseline corrected total area of the chromatogram and the average hydrocarbon response factor determined over the entire analytical range. Method reporting limits for total petroleum hydrocarbons and individual resolved alkanes in crude oils are 10,000 and 100 mg/kg, respectively; the detection limits are 100 mg/L and 10 ng/L, respectively, in water. Concentrations of hydrocarbons in oil are expressed on a weight/weight basis; those in water are expressed on a weight/volume basis. Given that seawater has a density of about 1.01 g/cm³, the error introduced by this convention is small.

Analysis of aromatic hydrocarbons and phenols

Each fresh oil and its three weathered fractions (when they could be obtained) and the stock WAFs prepared from them were analyzed by gas chromatography/mass spectrometry (GC/MS) for selected monocyclic aromatic hydrocarbons, polycyclic aromatic hydrocarbons, and phenols. The target alkanes, MAHs, PAHs, and phenols are summarized in the Appendix with method reporting limits.

The PAHs were quantified in the fresh and weathered oils and their WAFs by modifications of U.S. EPA Method 8270 [8,9]. The oils were extracted by diluting 50 mg, weighed to the nearest 0.01 mg, to 1 ml with a 1:1 dichloromethane:*n*-hexane mixture and spiking with low levels of the appropriate surrogate standards. The WAF samples were extracted three times with 60 ml of dichloromethane per liter of sample. Appropriate surrogate standards were added after addition of the first extraction solvent.

Prior to sample analysis, the GC/MS was tuned with perfluorotributylamine (PFTBA) and a minimum of a five-point initial calibration consisting of parent and alkylated PAH compounds was established to demonstrate the linear range of the analysis. Calibration check standards were analyzed with every

10 samples in order to monitor instrument response relative to the initial calibration. Quantitation of individual components was performed by the method of internal standards using relative response factors for individual components relative to the internal standards acenaphthene- d_{10} , phenanthrene- d_{10} , and benzo[a]pyrene- d_{12} . The PAH alkyl homologues were quantified with the straight baseline integration of each level of alkylation and the relative response factors of the respective parent PAH compound.

Sample extracts were analyzed with a Hewlett-Packard 5890 gas chromatograph equipped with a Hewlett-Packard 5970 mass selective detector operating in the selected ion monitoring mode. A 2- μ l aliquot of the sample was injected into a gas chromatograph equipped with a high resolution capillary column (J&W fused silica DB-5-MS, 30-m, 0.25-mm i.d., and 0.25- μ m film thickness) operated in the splitless mode. The temperature program and capillary column specification achieve near-baseline separation of the petroleum PAHs. The method detection limits for individual PAHs in crude oil and water are 0.2 mg/kg and 1 ng/L, respectively.

The analysis of the fresh and weathered oils and their WAFs for target MAHs was by a purge and trap method coupled to quantitation by GC/MS, a modification of U.S. EPA Method 8240. The MAH analysis by GC/MS was performed on a 50-m Petrocol capillary column (J&W Scientific, Folsom, CA, USA). Prior to sample analysis, the instrument was tuned with perfluorotributylamine and a minimum three-point calibration was performed to demonstrate the linear range of the analysis. The calibration solution is composed of selected paraffin, isoparaffin, aromatic, naphthene, and olefin compounds. Thirty-two target MAHs from benzene through several C_4 -benzene isomers were quantified in oil and WAF samples. Method detection limits for MAHs in crude oil and water are 100 mg/kg and 0.2 mg/L, respectively.

Analysis of the fresh and weathered oils and their WAFs for target phenol compounds was performed by GC/MS methods that are a modification of U.S. EPA Method 8270. Prior to sample analysis, the instrument was tuned with perfluorotributylamine and a minimum three-point calibration was performed to demonstrate the linear range of the analysis. The calibration solution was composed of selected phenols and quantitation of individual components was accomplished using the method of internal standards. Target phenols included phenol, total C_1 - through C_6 -alkylphenols, and selected individual alkylphenols (Appendix). Method reporting limits for phenols are similar to those for PAHs.

Preparation of the water-accommodated fraction

The water-accommodated fraction of the four oils and their weathered fractions was prepared by a low energy mixing technique [10,11]. Oil was layered on the surface of seawater in a glass container and the water was mixed gently for 60 h. Hydrocarbons partitioned from the oil into the water phase in relation to their relative affinities (solubilities) for the two phases, which can be defined by an oil/water partition coefficient.

A WAF was used in this investigation to simulate the types of dissolved and colloidal (nely dispersed) oil that might be encountered in the water column under an oil slick. Two types of WAF can be used, a single-ratio test (used in this investigation) and a multiple-ratio test [12]. In the single-ratio WAF preparation method, a single stock WAF is prepared by mixing oil and water at a single oil/water volume ratio for a set period

of time. The aqueous phase (the WAF) is decanted and diluted to produce a series of treatments containing different concentrations of the WAF. In the multiple-ratio method, several WAFs are prepared with different oil/water ratios. These WAFs are used for the exposure series in the bioassay. The single-ratio method produces test media containing hydrocarbons in the same proportions but at different concentrations; the multiple-ratio method produces WAFs with different relative concentrations of hydrocarbons due to depletion of MAHs at low oil/water ratios [11,13]. The single-ratio method was used in this investigation to facilitate interpretation of the dose/response relations of the WAFs to their hydrocarbon and phenol compositions.

The loading rate used in this investigation for WAF preparation was 28 g/L of oil in seawater. To prepare WAFs, an acid-washed, acetone-rinsed, glass 20-L carboy was placed on a magnetic stirrer and the appropriate volume of 0.45- μ m filtered natural seawater was added. A Teflon-coated stir bar was placed in each carboy, and the stirring rate was calibrated to three to four revolutions per second. Oil was weighed and transferred to the water in the carboy in acid-washed, solvent-rinsed glass jars. Residual oil in the glass jars was rinsed with seawater into the carboy. The carboy was then sealed with a Teflon-lined stopper and stirred in the dark for 60 h. No vortex was allowed during stirring to prevent dispersion of oil droplets into the aqueous phase.

The water-accommodated fraction was siphoned from the bottom of the carboy approximately 1 h after stirring was terminated. To ensure that WAF was not contaminated with the oil remaining on the water surface, the siphon was lowered into the carboy covered by a sealed, removable glass sleeve. Serial dilutions of WAFs were prepared in separate 5-gallon (19-L) aquaria by diluting the 100% WAF with the appropriate amount of 0.45- μ m filtered natural seawater. Each test chamber was then placed on a water table to adjust to test conditions.

The WAF samples for MAH analysis were placed in 40-ml volatile organic analyte bottles, leaving no head space, and stored at 4°C. The WAF samples for alkane, phenol, and PAH analysis were placed in 2.4-L amber glass bottles and preserved with ultrapure dichloromethane.

Test animals

Three species of tropical/subtropical marine animals and three species of temperate marine animals were used in the acute toxicity tests with the four oils. The tropical/subtropical species were clownfish, *Amphiprion clarkii*; tropical penaeid shrimp, *Penaeus vannamei*; and sea urchin larvae, *Arbacia punctulata*. The temperate species were silverside minnows, *Menidia beryllina*; mysids, *Americamysis (Mysidopsis) bahia*; and sea urchin larvae, *Strongylocentrotus purpuratus*, or sand dollar larvae, *Dendraster excentricus*. *Dendraster excentricus* larvae were used when *S. purpuratus* larvae were unavailable.

All the test species except the sand dollars were purchased from commercial suppliers. Adult sand dollars were collected from Sequim Bay, Washington, USA. All test species were acclimated to test conditions in running natural seawater in the laboratory for several days before the tests and were fed foods appropriate for each species during holding.

Performance of toxicity tests

All tests were performed with serial dilutions of 0, 8, 16, 64, and 100% WAF, with the exception of some tests with *A.*

clarkii and *A. punctulata* that were performed with 0, 8, 16, and either 75 or 100% WAF dilutions. Water-only, reference toxicant tests with copper sulfate were performed concurrently with each toxicity test to provide an indication of test-animal health and sensitivity.

Toxicity tests with *P. vannamei*, *A. bahia*, *M. beryllina*, and *A. clarkii* were very similar. All four toxicity tests were performed as 96-h, static-acute bioassays according to American Society for Testing Materials procedures [14]. Both the *P. vannamei* and *M. beryllina* tests were performed in 300 ml of test solution in 500-ml glass jars. The *A. bahia* tests were performed in 200 ml of test solution in 300-ml glass jars. The *A. clarkii* tests were performed in 1.7 L of test solution in 2-L glass jars. In each test, three replicates of each dilution were tested for each exposure concentration. Each test chamber contained 10 animals (3 for clownfish) for a test population of 50 (15 for clownfish) test organisms per treatment.

Water quality parameters (temperature, dissolved oxygen, pH, and salinity) were measured in all replicates prior to test initiation, in at least one replicate daily, and in all replicates at test termination [14]. Water quality parameters were maintained within acceptable ranges for each species during the tests.

Total ammonia concentrations were measured with a Fischer Accumet 1003 meter (Fisher Scientific, Fairlawn, NJ, USA) fitted with an ammonia-ion-specific probe in water samples collected from the *A. clarkii* tests before test termination. Observations were made daily of number of animals alive, dead, and moribund, and dead animals were removed. Test organisms, except clownfish, were fed brine shrimp *nauplii* daily. Clownfish were not fed during tests. At the end of the 4-d period, termination counts of number alive, dead, or missing were recorded. As a quality control check, a second observer confirmed surviving test organisms on at least 10% of the termination counts.

Tests with larval echinoderms were performed as 60-h, static-acute tests according to American Society for Testing and Materials procedures [15]. Embryo stocking densities were between 23 and 30 embryos/ml. Water quality measurements were recorded at test initiation in the stock solutions and at termination in one replicate container of each treatment. Water quality values were maintained within acceptable ranges for each species during tests.

The larval test was terminated after 60 h, when greater than 90% of the larvae in the seawater-only controls had completed development to the pluteus stage. One milliliter of 50% formaldehyde in seawater was added to each test chamber to terminate the test. Samples were scored for the appearance of normal pluteus larvae and abnormal larvae (abnormal pluteus larvae and developmentally delayed/developmentally delayed-abnormal larvae).

Statistical analysis

A median lethal concentration (LC50) or median effects concentration (EC50) was calculated for each treatment dilution series. In this study, the EC50 is the concentration that produces abnormal development in 50% of the test population of echinoderm larvae. The LC50/EC50 and its 95% confidence intervals were calculated by the trimmed Spearman-Kärber method [16], a modified regression analysis. The LC50s and EC50s were calculated based on % WAF. The LC50s and EC50s also were estimated based on measured concentrations of individual and total MAHs, PAHs, and phenols in the WAFs.

Table 1. Density (at 20°C) and evaporative weathering behavior of four Australian Northwest Shelf oils and their weathered fractions

Crude oil	Topped fraction	Density (g/cm ³)	Volume percent distilled	Weight percent residue
Campbell condensate	Fresh oil	0.754	0	100
	150°C 1	0.804	66.5	32.5
	200°C 1	0.831	88.4	11.1
	250°C 1	NO ^a	NO	NO
Wonnich crude	Fresh oil	0.775	0	100
	150°C 1	0.831	68.3	35.4
	200°C 1	0.844	78.0	23.8
	250°C 1	NO	NO	NO
Agincourt crude	Fresh oil	0.800	0	100
	150°C 1	0.849	36.5	65.1
	200°C 1	0.871	53.8	49.1
	250°C 1	0.895	71.3	32.1
Australian diesel fuel	Fresh oil	0.855	0	100
	150°C 1	NO	NO	NO
	200°C 1	0.856	3.1	96.8
	250°C 1	0.860	23.6	76.7

^a NO 5 fraction was not obtained.

RESULTS AND DISCUSSION

Oil weathering

The four fresh oils have densities at 20°C ranging from 0.754 to 0.855 g/cm³ (Table 1). These densities indicate that Campbell condensate and Wonnich crude oil are light crudes, Agincourt crude oil is a light- to middle-weight crude, and Australian diesel fuel is a typical middle distillate fuel. The density of the three crude oils increases by about 0.07 g/cm³ during weathering to 200°C. The density of the diesel fuel does not increase much during weathering.

Each of the four oils responds differently to evaporative weathering. There is not sufficient residue left after the 200°C 1 topping to prepare a 250°C 1 topped fraction from Campbell condensate and Wonnich crude oil. Nearly all the oil evaporates at 200°C 1. The boiling point range of the diesel fuel is so high that very little hydrocarbons are distilled off at 150°C 1; only 200°C 1 and 250°C 1 fractions of diesel fuel can be produced.

Evaporative weathering results in a substantial loss of the total mass of Wonnich and Campbell oils (Table 1); the oils evaporate nearly completely at a distillation temperature of 250°C 1. Agincourt crude oil is heavier; 32% of the original mass of the oil remains after distillation at 250°C 1. Middle distillate fuels, such as Australian diesel fuel, are composed of the distillation fraction boiling primarily between 175 and 375°C. This is reflected in the observation that nearly 77% of the diesel fuel remains after evaporative weathering at 250°C 1.

The distillation temperatures correspond approximately to weathering times on the sea surface at about 13°C sea and air temperature of 1 to 3 h (150°C 1), 0.5 to 1 d (200°C 1), and 2 to 5 days (250°C 1) [6]. Should one of these oils be released to surface waters of the Northwest Shelf, between about 11% (Campbell condensate) and 97% (Australian diesel fuel) would persist on the sea surface for a day under calm sea conditions (no wind or waves). After about a week or less, Campbell condensate and Wonnich crude oil would evaporate completely from the sea surface and the volume of the Australian diesel fuel slick would be reduced by about 23%. Surface water

Table 2. Hydrocarbon composition of three fresh crude oils and an Australian diesel fuel; concentrations are mg/kg

Chemical	Campbell	Wonnich	Agincourt	Diesel
Total resolved + unresolved hydrocarbons	602,000	469,000	783,000	814,000
Alkanes	170,000	134,000	117,000	224,000
Monocyclic aromatic hydrocarbons (MAHs)	121,000	148,000	12,500	10,800
Polycyclic aromatic hydrocarbons (PAHs)	10,100	13,400	5,320	40,100
C ₆ - to C ₉ -phenols	74	3,640	ND ^a	ND

^a ND = not detected.

temperatures on the Northwest Shelf range seasonally between 18 and 31°C; winds are moderate to strong (5±20 m/s) in the winter and moderate (5±10 m/s) in the summer [2]. Under these windy and higher water temperature conditions, evaporative weathering of the crude and refined oils would be more rapid than measured in the laboratory. Additional hydrocarbons would be lost from surface slicks by dissolution, which was not simulated here. Between 1 and 5% of the mass of a crude oil may dissolve from a sea-surface slick into the water column [5].

Composition of the oils

The four fresh oils differ in hydrocarbon composition (Table 2). Concentrations of total resolved plus unresolved hydrocarbons, measured by gas chromatograph with flame ionization detection, range from 469,000 to 814,000 mg/kg (47±81 weight%). The resolved alkanes include *n*-alkanes and selected isoalkanes between C₁₀ and C₃₆. The unmeasured petroleum components include hydrocarbons with boiling points less than that of octane (125°C) or greater than that of hexatriacontane (C₃₆) (320°C) and polar, nonhydrocarbon organic components of the oils. C₆- through C₉-alkanes are visible on the gas chromatograms but were not quantified because of low recoveries due to their volatility.

Concentrations of total resolved hydrocarbons and related compounds (alkanes, MAHs, PAHs, and phenols) are 17 to 64% of the concentrations of total resolved plus unresolved hydrocarbons, the %age decreasing with increasing oil weight. The unresolved components, sometimes called the unresolved complex mixture, are a complex mixture of branched and cyclic alkanes, naphtho-aromatic compounds, and resins [17]. Resolved alkanes are roughly as abundant as MAHs in the two light crude oils but are much more abundant than MAHs in Agincourt crude oil and Australian diesel fuel. Total PAHs are much less abundant than MAHs in the three crude oils but are more abundant than MAHs in the diesel fuel. Concentrations of total phenols are low in all but Wonnich crude oil. Agincourt crude oil is unusual in the relatively low concentrations in it of resolved alkanes, MAHs, and PAHs, possibly reflecting some biodegradation of the oil in the reservoir rocks [18].

During evaporative weathering of all the oils, there is a progressive loss of lower molecular weight C₁₀- through C₁₃-alkanes and benzene, naphthalene, phenol, and their C₁- and C₂-alkyl homologues. Concentrations of most higher molecular weight alkanes, MAHs, PAHs, and phenols increase. The increase in the concentrations of higher molecular weight, less volatile hydrocarbons in the oils during weathering is caused by the loss of the lower molecular weight, volatile hydrocarbons.

These patterns of change in the compositions of the crude oils during weathering may be somewhat different in a slick on the sea surface. The low molecular weight alkanes, phenols,

and aromatic hydrocarbons that are lost most rapidly by evaporative weathering also are the components of the oil that are the most water soluble. Their concentrations in a slick on the sea surface would decrease even faster than measured in this study due to the added loss from dissolution. Some of the higher molecular weight hydrocarbons, particularly the PAHs, would be lost by photolysis under the intense sunlight of the Northwest Shelf. A photolysis rate of 0.004%/d has been measured in a slick of light Arabian crude oil [19]. Biodegradation by indigenous microbiota also would contribute to the loss of some hydrocarbons from a slick on the sea surface and in the water column [4]. These weathering processes would go on simultaneously in surface slicks of all four oils.

Composition of the WAFs of the oils

The water-accommodated fractions of the four oils and their weathered fractions were analyzed for MAHs, phenols, and PAHs by GC/MS. The chemical composition of the WAFs reflects the compositions of the fresh and weathered crude oils and the relative seawater solubilities of the different hydrocarbons and phenols contained in the oils.

The WAFs of fresh Wonnich and Campbell crude oils contain 34.40 and 37.18 mg/L total MAHs, respectively. Nearly all the MAHs in the WAFs are benzene, toluene, and xylenes (Tables 3 and 4). Only very low concentrations of trimethylbenzenes are present. Most of the MAHs are lost from the oils during weathering; this is reflected in the low concentrations of these volatile hydrocarbons in the WAFs of the weathered oils. No MAHs are detected in the 150°C 1 fraction of the Wonnich crude oil WAF; they are present in the original WAF but probably were lost during sampling, shipping, or handling of the WAF. The WAFs of the 200°C 1 fractions of Wonnich crude oil and Campbell condensate contain 2.79 and 1.29 mg/L total MAHs, respectively; only traces of benzene, toluene, and ethylbenzene are present (Table 3).

Total phenol concentrations in the WAFs of fresh Campbell condensate and Wonnich crude oil are 1.42 and 0.95 mg/L, respectively (Tables 3 and 4). Phenol concentrations in the WAFs are less affected than concentrations of MAHs by weathering. The most abundant phenols in the WAFs are C₁- through C₄-phenols. Concentrations of phenol and cresols decrease and concentrations of C₃- and C₄-phenols increase in the WAFs as a result of weathering of the oils.

Concentrations of total PAHs in the WAFs of the two light crude oils are 0.45 (Wonnich) and 0.18 mg/L (Campbell). Weathering of the two oils results in an increase in the concentrations of total and most individual PAHs in their WAFs (Tables 3 and 4). The WAFs of the 200°C 1 fractions of Wonnich and Campbell crude oils contain 1.48 and 0.73 mg/L total PAHs, respectively. The most abundant PAHs in the WAFs of the two oils and their weathered fractions are naphthalene and alkyl naphthalenes. No PAHs with molecular weights higher

Table 3. Concentrations of monocyclic aromatic hydrocarbons (MAHs), polycyclic aromatic hydrocarbons (PAHs), and phenols in the 100% water-accommodated fractions (WAFs) of fresh and weathered Wonnich crude oil; only compounds that were detected at \$0.0001 mg/L in one or more samples are included; concentrations are mg/L (parts per million)

Chemical	Fresh oil WAF	1508C 1 ^a WAF	2008C 1 WAF
Benzene	13.33	ND	0.001
Toluene	14.80	ND	ND
Ethylbenzene	0.60	ND	ND
Xylenes	5.33	ND	1.93
C ₃ -benzenes	0.33	ND	0.72
C ₄ -benzenes	ND	ND	0.13
Total MAHs	34.40	ND	2.79
Naphthalene	0.26	0.70	0.67
C ₁ - to C ₃ -naphthalenes	0.18	0.63	0.76
Biphenyl	0.004	0.02	0.02
Acenaphthene	ND ^b	0.002	0.002
Dibenzofuran	ND	0.003	0.003
Fluorene	0.002	0.008	0.01
C ₁ - to C ₃ -fluorenes	ND	ND	0.07
Anthracene	ND	0.001	0.002
Phenanthrene	ND	0.003	0.003
C ₁ - to C ₃ -phenanthrenes	ND	ND	0.005
Total PAHs	0.45	1.36	1.48
Phenol	0.06	0.01	ND
C ₁ - to C ₅ -phenols	1.35	0.80	1.18
2- <i>r</i> -Butyl-3-methylphenol	ND	ND	0.003
Total phenols	1.42	0.81	1.18
Total analytes	36.27	2.17	5.45

^a No MAHs were detected because of a sampling/analysis error.

^b ND 5 not detected at \$0.0001 mg/L.

Table 4. Concentrations of monocyclic aromatic hydrocarbons (MAHs), polycyclic aromatic hydrocarbons (PAHs), and phenols in the 100% water-accommodated fractions (WAFs) of fresh and weathered Campbell condensate; only compounds that were detected at \$0.0001 mg/L in one or more samples are included; concentrations are mg/L (parts per million)

Chemical	Fresh oil WAF	1508C 1 WAF	2008C 1 WAF
Benzene	15.33	0.47	0.04
Toluene	14.13	1.90	0.05
Ethylbenzene	0.08	0.10	0.03
Xylenes	6.61	7.93	0.59
C ₃ -benzenes	1.04	1.53	0.57
C ₄ -benzenes	ND	ND ^a	0.04
Total MAHs	37.18	11.93	1.29
Naphthalene	0.11	0.23	0.31
C ₁ - to C ₄ -naphthalenes	0.07	0.19	0.40
Biphenyl	0.002	0.005	0.009
Acenaphthene	0.0002	0.0005	0.001
Dibenzofuran	0.0002	0.0006	0.001
Fluorene	0.0006	0.002	0.004
C ₁ - to C ₃ -fluorenes	0.0003	0.001	0.004
Anthracene	ND	ND	0.0001
Phenanthrene	0.0001	0.0004	0.001
C ₁ - to C ₃ -phenanthrenes	0.0001	0.0005	0.001
Dibenzothiophene	ND	0.0001	0.0002
C ₁ - to C ₂ -dibenzothiophenes	ND	0.0002	0.0002
Total PAHs	0.18	0.43	0.73
Phenol	0.03	0.02	0.002
C ₁ - to C ₆ -phenols	0.92	1.65	0.89
Total phenols	0.95	1.67	0.89
Total analytes	38.31	14.03	2.91

^a ND 5 not detected at \$0.0001 mg/L.

than that of fluoranthene and pyrene are present at concentrations above the method detection limit 1 ng/L (parts per trillion) in the WAFs.

The WAFs of fresh and weathered Agincourt crude oil and Australian diesel fuel contain low concentrations of all three analyte groups (Tables 5 and 6). The 2008C 1 WAF of Agincourt crude oil contains higher concentrations of all analytes than expected based on the composition of the WAF of the fresh oil. This may have been caused by the presence of a microdroplet of oil in the water sample. The MAHs were not detected in the WAFs of fresh and 2508C 1 weathered Agincourt crude oil, probably because of an analytical error. Concentrations of MAH decrease from 0.12 to 0.09 mg/L in the WAFs of the 150 and 2008C 1 weathered fractions. The most abundant MAHs in the Agincourt WAFs are the C₂-, C₃-, and C₄-benzenes. Total PAH concentrations in the WAFs of fresh and weathered Agincourt crude oil range from 0.05 to 0.003 mg/L. The most abundant PAHs are alkyl naphthalenes and alkyl dibenzothiophenes. Total phenol concentrations range from 0.02 to 0.002 mg/L.

The MAH concentrations in the WAFs of Australian diesel fuel drop with weathering from 0.45 mg/L in the WAF of the fresh oil to 0.05 mg/L in the WAF of the 2508C 1 weathered fraction (Table 6). Phenols concentrations also decrease with weathering in the WAFs of Australian diesel fuel. However, PAH concentrations remain relatively constant in the WAFs, ranging from 0.12 to 0.29 mg/L. The PAH concentration in diesel fuel WAFs is much lower than in WAFs of fresh and weathered Campbell condensate and Wonnich crude oil despite the higher concentrations of PAHs in fresh and weathered diesel fuel. About half the PAHs in the diesel fuel are three- and four-ring compounds that do not partition well into the WAF.

Toxicity of the oil WAFs

The freshly prepared dilutions of the WAFs of selected oils were analyzed for MAHs and PAHs to confirm that actual dilutions agreed with the nominal dilutions. There was good agreement between the nominal concentrations of MAHs and PAHs in the dilutions and the measured concentrations. It is well known that hydrocarbons, particularly MAHs, evaporate rapidly from WAFs [10,13]. At least 90% of the C₀ through C₂-MAHs may evaporate during the toxicity tests. These compounds also are lost rapidly from the water column during an oil spill. Toxicities of oil WAFs are expressed in this paper as % WAF or the initial concentrations of hydrocarbons in the WAFs.

The acute toxicities (96-h LC50 or 60-h EC50) of the WAFs, expressed as % WAF, of the four fresh and weathered oils and six species of marine animals vary widely (Table 7). Wonnich condensate and Campbell crude oil WAFs are the most toxic to all species, followed by WAFs of Australian diesel fuel. Agincourt crude oil WAFs are practically nontoxic. The LC50 or EC50 values range from 11% WAF (fresh Wonnich WAF and *Dendroaster* larvae) to >100% WAF.

The WAFs of fresh Wonnich crude oil and Campbell condensate have similar acute toxicities (30±48% WAF) for clownfish, silversides, mysids, and shrimp (Table 7). *Arbacia* larvae are about as sensitive to the WAF of fresh Wonnich crude oil but less sensitive to the WAF of fresh Campbell condensate than the two fish and two crustacean species. *Dendroaster* larvae are more sensitive than the fish, crustaceans, and the other echinoderm larvae to the WAF of fresh Wonnich

Table 5. Concentrations of monocyclic aromatic hydrocarbons (MAHs), polycyclic aromatic hydrocarbons (PAHs), and phenols in the 100% water-accommodated fractions (WAFs) of fresh and weathered Agincourt crude oil; only compounds that were detected at ≥ 0.0001 mg/L in one or more samples are included; concentrations are mg/L (parts per million)

Chemical	Fresh oil WAF ^a	1508C 1 WAF	2008C 1 WAF	2508C 1 WAF
Toluene	ND	ND	0.0006	ND
Ethylbenzene	ND	ND	0.0008	ND
Xylenes	ND	0.05	0.001	ND
C ₃ -benzenes	ND	0.05	0.04	ND
C ₄ -benzenes	ND	0.02	0.04	ND
Total MAHs	ND	0.12	0.09	ND
Naphthalene	ND ^b	ND	0.0001	ND
C ₁ - to C ₄ -naphthalenes	0.0001	0.0002	0.032	0.0006
Biphenyl	ND	ND	0.0008	ND
Acenaphthene	ND	ND	0.0009	ND
Dibenzofuran	ND	ND	0.0005	ND
Fluorene	ND	ND	0.002	ND
C ₁ - to C ₃ -fluorenes	0.0004	0.0005	0.002	0.001
Anthracene	ND	ND	0.0002	ND
Phenanthrene	ND	ND	0.002	0.0001
C ₁ - to C ₄ -phenanthrenes	0.0008	0.0009	0.002	0.002
Dibenzothiophene	ND	ND	0.0001	0.0001
C ₁ - to C ₄ -dibenzothiophenes	0.002	0.002	0.003	0.003
Pyrene	ND	ND	0.0001	ND
C ₁ - to C ₂ -fluoranthene/pyrenes	ND	0.0001	0.0003	0.0004
Total PAHs	0.003	0.003	0.05	0.007
C ₂ - to C ₆ -phenols	0.005	0.005	0.02	0.002
Total analytes	0.008	0.13	0.16	0.009

^a No MAHs were detected because of a sampling/analysis error.

^b ND = not detected at ≥ 0.0001 mg/L.

crude oil but are about as sensitive as the fish and crustaceans to the WAF of fresh Campbell crude oil. *Arbacia* and *Dendraster* larvae are more sensitive to the WAF of fresh Wonnich crude than the WAF of fresh Campbell condensate. Overall, the six marine animals are slightly more sensitive to Wonnich crude oil WAFs than to Campbell condensate WAFs. The tropical/subtropical marine animals (clownfish, shrimp, and *Arbacia*) are about as sensitive as the temperate species to the WAFs of fresh and weathered Northwest Shelf crude oils.

The fresh WAF of Agincourt crude oil is slightly toxic to silverside minnows and shrimp. The WAFs of weathered fractions of Agincourt crude oil are practically nontoxic to all but the mysids (Table 7). The three WAFs of the weathered fractions of the crude oil are about equally toxic to the mysids but are not acutely toxic to the other five species of test animals.

Water-accommodated fractions of fresh and weathered Australian diesel fuel are about as toxic as WAFs of the light crude oils, Wonnich crude and Campbell condensate, to silverside minnows, mysids, shrimp, and *Dendraster* larvae (Table 7). There is little change in the toxicity of the diesel fuel WAFs with weathering.

The effect of weathering on the toxicity of the oils is different for the three major taxonomic groups used in the bioassays (Table 7). Weathering of Wonnich crude oil and Campbell condensate progressively decreases the toxicity of their WAFs to the two fish species and *Dendraster* larvae. This pattern is present but less pronounced for WAFs of diesel fuel and silverside minnows and *Dendraster* larvae. Weathering has little effect on the toxicity of the WAFs of the oils to mysids, shrimp, and *Arbacia* larvae.

The LC50s and EC50s of WAFs of the four oils also can be expressed as concentrations of total MAHs, phenols, and PAHs (Table 8). At the LC50/EC50 concentrations, WAFs of

fresh Wonnich crude oil and Campbell condensate contain much higher concentrations of total MAHs than WAFs of their weathered fractions. Toxic concentrations to the six test species of WAFs of fresh Wonnich crude oil and Campbell condensate contain means of 11.5 \pm 6.4 and 15.1 \pm 6.7 mg/L, respectively, of MAHs. Mean concentrations of MAHs in acutely toxic WAFs of weathered Wonnich crude oil and Campbell condensate are lower, ranging from 3.04 \pm 3.39 mg/L for the WAF of the 2008C 1 fraction of Wonnich crude oil to 7.82 \pm 2.7 mg/L for the WAF of the 1508C 1 and 0.44 \pm 0.01 mg/L for the 2008C 1 fraction of Campbell condensate. Acutely toxic concentrations of WAFs of fresh diesel fuel contain 0.16 \pm 0.04 mg/L MAH. The WAFs of weathered fractions of Agincourt crude oil and Australian diesel fuel contain low concentrations of MAHs (0.02 \pm 0.27 mg/L).

Phenol and PAH concentrations show the opposite trend, being low at the LC50 concentrations of the WAFs of the fresh oils and increasing slightly at the LC50 concentrations of the WAFs of the weathered fractions (Table 8). The mean PAH concentrations in the acutely toxic concentrations of WAFs of fresh and weathered Wonnich crude oil increase from 0.15 \pm 0.05 mg/L for the fresh oil WAF to 0.90 \pm 0.68 mg/L for the WAF of the 2008C 1 fraction. The mean concentrations of PAHs in the acutely toxic concentrations of WAFs of Campbell condensate increase only slightly from 0.18 \pm 0.23 mg/L for the WAF of the fresh oil to 0.26 \pm 0.14 mg/L for the WAF of the 1508C 1 fraction and 0.21 \pm 0.01 mg/L for the WAF of the 2008C 1 fraction of Campbell condensate. The total phenols concentrations in acutely toxic WAFs of fresh and weathered Wonnich crude oil increase from 0.47 \pm 0.17 mg/L in the WAF of the fresh oil to 0.68 \pm 0.25 mg/L in the WAF of the 2008C 1 fraction. The mean total phenols concentration in the WAFs of fresh and weathered Campbell con-

Table 6. Concentrations of monocyclic aromatic hydrocarbons (MAHs), polycyclic aromatic hydrocarbons (PAHs), and phenols in the 100% water-accommodated fractions (WAFs) of fresh and weathered Australian diesel fuel; only compounds that were detected at ≥ 0.0001 mg/L in one or more samples are included; concentrations are mg/L (parts per million)

Chemical	Fresh WAF	2008C 1 WAF	2508C 1 WAF
Benzene	ND ^a	0.006	0.001
Toluene	0.001	0.001	0.003
Ethylbenzene	ND	ND	0.002
Xylenes	0.18	0.08	0.006
C3-benzenes	0.13	0.12	0.02
C4-benzenes	0.14	0.11	0.02
Total MAHs	0.45	0.32	0.05
Naphthalene	0.001	0.0004	0.0003
C ₁ - to C ₄ -naphthalenes	0.11	0.08	0.15
Biphenyl	0.01	0.008	0.007
Acenaphthene	0.001	0.001	0.002
Dibenzofuran	0.005	0.004	0.007
Fluorene	0.007	0.006	0.01
C ₁ - to C ₃ -fluorenes	0.007	0.008	0.04
Anthracene	0.0002	0.0002	0.0006
Phenanthrene	0.005	0.004	0.008
C ₁ - to C ₄ -phenanthrenes	0.006	0.006	0.05
Dibenzothiophene	0.0005	0.0004	0.001
C ₁ - to C ₄ -dibenzothiophenes	0.002	0.002	0.01
Fluoranthene	ND	ND	0.0002
Pyrene	0.0001	0.0001	0.0008
C ₁ - to C ₃ -fluoranthene/pyrenes	0.0002	0.0002	0.003
Chrysene	ND	ND	0.0001
C ₁ -chrysenes	ND	ND	0.0001
Total PAHs	0.16	0.12	0.29
Phenol	0.0006	0.0001	ND
C ₁ - to C ₇ -phenols	0.59	0.41	0.25
Total phenols	0.59	0.41	0.25
Total analytes	1.20	0.85	0.59

^a ND = not detected at ≥ 0.0001 mg/L.

condensate increase from 0.24 \pm 0.26 mg/L in the WAF of the fresh condensate to 1.04 \pm 0.36 mg/L in the WAF of the 1508C 1 fraction. Mean PAH and phenols concentrations in acutely toxic concentrations of WAFs of Agincourt crude oil and Australian diesel fuel are low and do not vary much with oil weathering.

These results suggest that MAHs contribute most to the toxicity of the WAFs of the fresh oils but are less important in the WAFs of the weathered fractions. Phenols and PAHs may not be important contributors to the toxicity of WAFs of the fresh oils but are important contributors to the toxicity of the WAFs of the weathered oils.

To evaluate this hypothesis further, we compared the estimated median lethal concentrations of the individual MAHs, PAHs, and phenols in the WAFs to their measured exposure concentrations in the 100% WAFs. The acute toxicity of individual MAHs and PAHs to aquatic animals was estimated by the regression of McCarty et al. [20],

$$\log \text{LC50 (mM)} = 5.20.90 \log K_{ow} - 1.71$$

The acute toxicity of phenols was estimated with the uncorrected (for ionization) regression of McCarty et al. [21] for polar narcotics,

$$\text{LC50 (mM)} = 5.20.55 \log K_{ow} - 1.064$$

where K_{ow} is the octanol/water partition coefficient. This LC50/ $\log K_{ow}$ relationship seems to be valid for nonpolar and slightly polar organic chemicals with $\log K_{ow}$ from about 1.5 to 6.0. The toxicities of the individual chemicals in the WAFs are assumed to be additive [20,21]. The hazard quotient for each analyte was calculated for each WAF as the concentration of the analyte in the 100% WAF divided by the estimated LC50. Hazard quotients were summed for each analyte group (MAHs, phenols, PAHs) to produce an estimate of the acute toxicity (the hazard index: HI) of each WAF. If the sum of the HIs for the three analyte groups in the WAFs was substantially less than one, we concluded that other, uncharacterized components of the WAFs contributed to their toxicity.

Estimated LC50s for individual aromatic hydrocarbons and phenols range from 61.4 mg/l (phenol) to 0.02 mg/L (C₁-chrysenes) (Table 9) and are in reasonable agreement with empirically determined aquatic LC50s for compounds of these types for which acute toxicity data are available [22,23]. However, as indicated in Tables 7 and 8, acute toxicities of these WAFs vary for different species, reflecting the different sensitivities of the different marine animals to the hydrocarbons in crude oil WAFs. The relative sensitivities of the different species to a particular WAF are similar (Table 8), suggesting that the

Table 7. The acute toxicity (96-h LC50 for fish and crustaceans and 60-h EC50 for echinoderm larvae), expressed as percent water-accommodated fraction (WAF), of the WAFs of three crude oils and an Australian diesel fuel and their weathered fractions to six species of marine animals

Oil fraction	Clownfish	Silverside	Mysid	Shrimp	Urchin ^a	Echinoderm ^b
Wonnich crude	35	32	48	30	44	11
Wonnich 1508C 1	69	79	43	38	37	24
Wonnich 2008C 1	100	72	32	35	31	61
Campbell condensate	39	37	36	30	68	33
Campbell 1508C 1	81	89	38	38	80	100
Campbell 2008C 1	100	100	35	33	100	100
Agincourt crude	100	88	100	80	100	100
Agincourt 1508C 1	100	100	78	100	100	100
Agincourt 2008C 1	100	100	84	100	100	100
Agincourt 2508C 1	100	100	79	100	100	100
Australian diesel	100	54	30	31	100	27
Diesel 2008C 1	100	79	32	31	100	100
Diesel 2508C 1	100	68	32	34	100	54

^a *Arbacia punctulata* larvae.

^b Urchin *Strongylocentrotus purpuratus* or sand dollar *Dendraster excentricus* larvae.

Table 8. Concentrations of total monocyclic aromatic hydrocarbons (MAHs), polycyclic aromatic hydrocarbons (PAHs), and phenols in the water-accommodated fractions (WAFs) of four Australian oils and their weathered fractions at the LC50/EC50 concentrations; concentrations are mg/L

Oil WAF	Species	LC50/EC50 (%WAF)	MAHs	PAHs	Phenols
Fresh Wonnich	Clownfish	35	12.04	0.16	0.50
	Silverside	32	11.01	0.14	0.45
	Mysid	48	16.51	0.21	0.68
	Shrimp	30	10.32	0.13	0.42
	<i>Arbacia punctulata</i> larvae	44	15.14	0.20	0.62
	<i>Dendraster excentricus</i> larvae	11	3.78	0.05	0.16
Wonnich 1508C 1	Clownfish	69	ND ^a	0.94	0.56
	Silverside	79	ND	1.08	0.64
	Mysid	43	ND	0.58	0.35
	Shrimp	38	ND	0.52	0.31
	<i>A. punctulata</i> larvae	37	ND	0.50	0.30
	<i>D. excentricus</i> larvae	24	ND	0.33	0.19
Wonnich 2008C 1	Silverside	72	2.01	1.06	0.85
	Mysid	32	0.89	0.47	0.38
	Shrimp	35	9.76	0.52	0.41
	<i>A. punctulata</i> larvae	31	0.86	0.46	0.37
	<i>D. excentricus</i> larvae	61	1.70	0.90	0.72
Fresh Campbell	Clownfish	39	14.50	0.07	0.290
	Silverside	37	13.76	0.07	0.03
	Mysid	36	13.39	0.07	0.27
	Shrimp	30	11.16	0.05	0.22
	<i>A. punctulata</i> larvae	68	25.29	0.12	0.50
	<i>D. excentricus</i> larvae	33	12.27	0.06	0.24
Campbell 1508C 1	Clownfish	81	9.74	0.42	1.29
	Silverside	89	10.71	0.05	1.42
	Mysid	38	4.57	0.20	0.60
	Shrimp	38	4.57	0.20	0.60
	<i>A. punctulata</i> larvae	80	9.62	0.42	1.27
Campbell 2008C 1	Mysid	35	0.46	0.22	0.32
	Shrimp	33	0.43	0.20	0.30
Fresh Agincourt	Silverside	88	ND	0.002	0.004
	Shrimp	80	ND	0.002	0.003
Agincourt 1508C 1	Mysid	78	0.10	0.003	0.004
Agincourt 2008C 1	Mysid	84	0.08	0.04	0.02
Agincourt 2508C 1	Mysid	79	ND	0.006	0.003
Fresh diesel	Silverside	54	0.24	0.09	0.32
	Mysid	30	0.13	0.05	0.18
	Shrimp	31	0.14	0.05	0.18
	<i>D. excentricus</i> larvae	27	0.12	0.04	0.16
Diesel 2008C 1	Silverside	79	0.27	0.09	0.33
	Mysid	32	0.11	0.04	0.13
	Shrimp	31	0.11	0.04	0.13
Diesel 2508C 1	Silverside	68	0.05	0.20	0.17
	Mysid	32	0.02	0.09	0.08
	Shrimp	34	0.02	0.10	0.08
	<i>D. excentricus</i> larvae	54	0.04	0.16	0.14

^a ND = not detected.

relative sensitivities to different components of the WAFs are similar. If it is assumed that the relative toxicities of different hydrocarbons in a WAF are the same for different species, then the estimated LC50 values can be used to estimate the contribution of different groups of petroleum components to the observed toxicity of the WAFs (Table 10).

The WAFs of fresh and weathered Wonnich crude oil and Campbell condensate have total HIs greater than one, with two exceptions. The WAF of the 1508C 1 fraction of Wonnich crude oil would have a HI greater than one if the MAHs were included. However, they were not quantified because of an analytical error. The WAF of the 2008C 1 fraction of Campbell condensate has a HI of 0.73, due mainly to loss of most MAHs.

The MAHs represent 86 and 95% of the total HIs of the two fresh oil WAFs and 34 to 86% of the total HIs of the WAFs of the weathered fractions.

Water-accommodated fractions of fresh and weathered Agincourt crude oil contain low concentrations of MAHs and PAHs (Table 5), have a low acute toxicity, and have HIs well below one. The MAHs were not detected in the WAFs of fresh and the 2508C 1 fraction of Agincourt crude oil due to an analytical error. However, MAH concentrations in fresh and weathered Agincourt crude oil and Australian diesel fuel are similar (Table 2), indicating that the WAF of fresh Agincourt crude oil probably contains about 0.5 mg/L MAHs. If this is the case, MAHs are the major contributors (about 80%) to the

Table 9. Estimated acute toxicities of monocyclic aromatic hydrocarbons (MAHs), polycyclic aromatic hydrocarbons (PAHs), and phenols that were found in the water-accommodated fractions (WAFs) of Wonnich crude oil and Campbell condensate, based on the log LC50/log K_{ow} regressions of McCarty et al. [20,21] for nonpolar and polar narcotics

Chemical	Molecular weight	Log K_{ow}	LC50 (mg/L)
Benzene	78.1	2.13	48.42
Toluene	92.1	2.65	19.34
Ethylbenzene	106.2	3.13	8.28
Xylenes	106.2	3.18	7.43
C ₃ -benzenes	120.2	3.58	3.73
C ₄ -benzenes	134.2	4.0	1.74
Naphthalene	128.2	3.37	6.10
C ₁ -naphthalenes	142.2	3.87	2.40
C ₂ -naphthalenes	156.2	4.37	0.93
C ₃ -naphthalenes	170.2	5.0	0.28
C ₄ -naphthalenes	184.2	5.55	0.10
Biphenyl	154.2	3.95	2.20
Acenaphthene	154.2	3.92	2.34
Dibenzofuran	168.2	4.21	1.40
Fluorene	166.2	4.18	1.47
C ₁ -uorenes	180.2	4.97	0.31
C ₂ -uorenes	194.2	5.2	0.21
C ₃ -uorenes	208.2	5.5	0.12
Anthracene	178.2	4.54	0.75
Phenanthrene	178.2	4.57	0.70
C ₁ -phenanthrenes	192.3	5.14	0.23
C ₂ -phenanthrenes	206.3	5.51	0.12
Dibenzothiophene	184.3	4.49	0.86
C ₁ -dibenzothiophenes	198.3	4.86	0.43
C ₂ -dibenzothiophenes	212.3	5.5	0.12
C ₃ -dibenzothiophenes	233.3	5.73	0.08
Fluoranthene	202.3	5.22	0.20
Pyrene	202.3	5.18	0.23
C ₁ -uoranthene/pyrenes	216.1	5.72	0.08
Chrysene	228.3	5.86	0.06
C ₁ -chrysenes	242.1	6.42	0.02
Phenol	94.1	1.5	61.44
C ₁ -phenols	108.1	1.98	38.49
C ₂ -phenols	122.2	2.35	27.24
C ₃ -phenols	136.2	2.70	19.48
C ₄ -phenols	150.2	3.31	9.91
C ₅ -phenols	164.2	3.5	8.54
C ₆ -phenols	178.3	3.6	8.20

Table 10. Hazard indices (HI) for monocyclic aromatic hydrocarbons (MAHs), polycyclic aromatic hydrocarbons (PAHs), and phenols in water-accommodated fractions (WAFs) of four fresh and weathered oils from Australia; HIs are the sum of the hazard quotients (concentration in 100% WAF/acutely lethal concentration) for each analyte type

Oil WAF	MAH	PAH	Phenols	Total HI
Fresh Wonnich	2.57	0.29	0.14	3.00
Wonnich 1508C 1	ND ^a	0.58	0.08	0.66
Wonnich 2008C 1	0.53	0.93	0.12	1.58
Fresh Campbell	2.23	0.07	0.05	2.35
Campbell 1508C 1	1.60	0.18	0.08	1.86
Campbell 2008C 1	0.26	0.42	0.05	0.73
Fresh Agincourt	ND	0.03	, 0.001	0.03
Agincourt 1508C 1	0.03	0.04	, 0.001	0.07
Agincourt 2008C 1	0.03	0.14	0.002	0.17
Agincourt 2508C 1	ND	0.06	, 0.001	0.06
Fresh diesel	0.12	0.30	0.07	0.49
Diesel 2008C 1	0.11	0.24	0.05	0.40
Diesel 2508C 1	0.02	0.77	0.03	0.82

^a ND 5 not detected because of an analytical error.

total HI (; 0.15) of the WAF to silversides and shrimp. The MAHs also contribute 17 to 42% to the total HI of the WAFs of weathered Agincourt crude oil. Water-accommodated fractions of fresh and weathered Australian diesel fuel contain low concentrations of MAHs (Table 6), and these aromatic hydrocarbons contribute less than 50% to the total HIs of the WAFs.

Polycyclic aromatic hydrocarbons are the most important contributors to the WAFs of the 2008C 1 fractions of Wonnich crude oil, Campbell condensate, and Agincourt crude oil and to fresh and weathered fractions of Australian diesel fuel. The PAHs account for 3 to 94% of the total estimated HIs of the WAFs of the fresh and weathered oils. The contribution of PAHs to the total HIs of the WAFs increases with weathering of the oils.

The HIs for PAHs in the WAFs of fresh and weathered oil fractions range from 0.03 to 0.93. It is uncertain whether HIs for PAHs would exceed one with further evaporative weathering of the WAFs. Most of the PAHs that are likely to be lost from the weathered oils by evaporation and dissolution have been lost from the 2508C 1 weathered fractions; therefore, it is unlikely that the trend of increasing contribution of PAHs to WAF toxicity would continue indefinitely during further weathering. Toxicity of PAHs increases with molecular weight (Table 9); the most persistent PAHs have the highest toxicities. However, concentrations of high molecular weight PAHs (above phenanthrene) are low in crude oils and their oil/water partition coefficients are so high that only traces of these compounds are present in oil WAFs. Although these high molecular weight PAHs are relatively persistent in weathered crude oil, they probably do not contribute much to the toxicity of dissolved and finely dispersed oil in the water column.

Phenols, because of their lower toxicities than aromatic hydrocarbons of similar molecular weight and their relatively low concentrations in the WAFs, have HIs of , 0.001 to 0.14. They contribute less than 1 to 14% to the total HIs of the WAFs of the four oils and their weathered fractions.

The HI values for the WAFs of the fresh and weathered oils are in reasonable agreement with the relative toxicity of the WAFs to the six species of marine animals. The WAFs of fresh Wonnich crude oil and Campbell condensate are the most toxic, followed by the WAFs of weathered fractions of Wonnich crude oil. Water-accommodated fractions of Agincourt crude oil are practically nontoxic. The toxicity of WAFs of fresh and weathered Australian diesel fuel is greater than predicted from HI values.

With one exception, total HIs for WAFs of fresh and weathered Agincourt crude oil and Australian diesel fuel are well below one, suggesting that unanalyzed components of the WAFs contribute to their toxicity. The WAFs of fresh and weathered Agincourt crude oil contain 0.03 to 0.07 mg/L total resolved C₁₄ through C₃₅ n-alkanes and isoprenoids and 2.0 to 2.5 mg/L total resolved plus unresolved hydrocarbons. The WAFs of fresh and weathered Australian diesel fuel contain 0.03 to 0.08 mg/L total resolved C₁₄ through C₃₁ n-alkanes and isoprenoids and 2.7 to 3.6 mg/L total resolved plus unresolved hydrocarbons. The unresolved hydrocarbons include a complex mixture of branched and cyclic alkanes and naphthenoaromatic hydrocarbons, related hetero-compounds, and slightly polar compounds (resins) [17]. These unresolved hydrocarbons, particularly the low molecular weight, slightly soluble cyclic alkanes and resins, may contribute to the toxicity of these WAFs.

Barron et al. [24] recently evaluated the toxicity of WAFs

of three weathered middle distillate fuels to mysids. There was no relationship between the relative toxicities of the three WAFs and the concentrations in them of individual and total PAHs. The authors concluded that PAHs were not the major determinant of the toxicity of the three WAFs. Hokstad et al. [25] evaluated the chemical composition and Microtox[†] (Microsoft, Redmond, WA, USA) toxicity of WAFs of fresh and weathered fractions of four crude oils and a condensate. As in the present study, MAHs contributed most to the toxicity of the WAFs of the fresh oils, but the sources of the toxicity of the WAFs of the weathered oils was uncertain. The concentration of a soluble, polar fraction (acids and alcohols) increased in the WAFs with weathering. This polar fraction may have contributed to the toxicity of the WAFs of the weathered oils. The concentration of the polar fraction in the WAFs corresponded reasonably well with the concentration of the resin fraction in the fresh and weathered oils. Some resin components of the oils are water soluble and may have contributed to the toxicity of the WAFs. Diesel fuel often contains additives, added to improve the properties of the fuel under different meteorological conditions. These additives could have contributed to the toxicity of the WAFs of fresh and weathered Australian diesel fuel.

The patterns of changing contributions of different petroleum component groups to the toxicity of the WAFs of the fresh and weathered crude oils reflect the effects of evaporative weathering on the composition of the crude oils and their WAFs. The MAHs, particularly the benzene, toluene, ethylbenzene, and xylenes compounds, although less toxic than the PAHs, are so much more abundant in the WAFs of the fresh light crude oils that they contribute most to their toxicity.

With the loss of most of the low molecular weight MAHs, particularly benzene and toluene, during evaporative weathering, the PAHs become more important contributors to the toxicity of the WAFs (Table 10). Although there is some loss of light PAHs, such as naphthalene, methylnaphthalene, and toluene, during evaporative weathering, loss of other more volatile hydrocarbons is even greater, resulting in a net increase in the concentrations of all PAHs in the weathered oils and their WAFs (Tables 3 through 6). The increase in the concentrations of PAHs, coupled to the decreases in MAHs, in the WAFs with weathering of the oils causes the observed progressive increase in the contribution of the PAHs to the toxicity of the WAFs with weathering of the source oils. Concentrations of phenols in the WAFs of the fresh and weathered oils do not change much, and their contribution to the toxicity of the WAFs remains small.

The artificial weathering conditions used in this investigation simulate a spill under relatively mild conditions of low wind speed and wave action. Under these conditions, evaporation is primarily from a surface slick, and hydrocarbons go into solution in the underlying water column with gentle mixing. Concentrations of accommodated (dissolved and finely dispersed) hydrocarbons (the sum of MAHs, PAHs, and phenols) in WAFs (simulating the water column under a slick) of the oils decrease rapidly from about 0.5 to 38 mg/L initially (within the first hour after a spill) to 0.1 to 14 mg/L after about 2 h to 0.2 to 5 mg/L after about 1 d (Tables 4 to 6). Within a week, surface slicks of the two light crude oils will evaporate completely from the sea surface, leaving only traces of hydrocarbons, mostly PAHs, in the water column [26]. After about 1 week on the sea surface, less than 30 and 75% of Agincourt crude oil and Australian diesel fuel, respectively,

will remain, and total hydrocarbon concentrations in the underlying water column will be well below 1 mg/L.

The WAF can be considered to contain the highest possible concentrations of dissolved petroleum hydrocarbons expected from a spill under these conditions. In an actual spill situation, the oil to water ratio is much lower than that used to prepare the WAFs (about 33:1), and the dissolved hydrocarbon concentration in the water decreases as the oil to water ratio decreases [13]. Measured concentrations of total petroleum hydrocarbons under the slick of an experimental spill of 20 m³ of Norwegian Sture blend crude oil in the North Sea ranged from about 0.002 to 0.006 mg/L (Per Daling, IKU-SINTEF, Trondheim, Norway, personal communication). When the slick was dispersed with a chemical dispersant, total petroleum hydrocarbon concentrations in the water column increased to 0.5 to 0.85 mg/L and concentrations of BTEX were in the range of 0.005 to 0.008 mg/L. In an actual spill situation, some oil will be dispersed into the water column. The contribution of the dispersed oil droplets to the toxicity of the total oil (dissolved and dispersed) to water column organisms is small, probably 15% or less [26].

Following the *Exxon Valdez* crude oil spill in Prince William Sound, Alaska, USA, an extensive water quality program was performed and nearly 3,000 water column samples were analyzed for total PAHs [27]. The highest concentration of total PAHs measured in the upper water column near slicks shortly after the spill was 0.019 mg/L. Only 17% of water samples collected in spill-path areas contained more than 0.001 mg/L total PAHs. Wolfe et al. [28] estimated that the average concentration of total PAHs in the water column under the oil slick in Prince William Sound in the week immediately after the spill was about 0.012 mg/L. These measured and estimated concentrations were confirmed by estimating concentrations of PAHs in the water column needed to produce, by bioaccumulation, the concentrations of PAHs measured in tissues of fish and mussels from the spill-path area [29]. Therefore, concentrations of PAHs, and probably other petroleum hydrocarbons, in solution in the water column near even a massive crude oil spill are unlikely to exceed about 0.1 mg/L for more than a few hours after the start of the spill or chemical dispersion of the oil slick. If the release of petroleum is to the sea floor following a blowout, concentrations of benzene, toluene, ethylbenzene, and xylene may exceed 0.1 mg/L in the water column near the blowout, as occurred after the Ixtoc blowout in the Bay of Campeche, Mexico [30].

The results of this study have shown that, under mild conditions with little physical dispersion of petroleum into the water column, volatile monocyclic aromatic hydrocarbons dissolving from the surface slick would be the main contributors to any toxicity observed in water column organisms near the surface slick within a few hours to a day after the spill. After the oils weathered on the sea surface for more than about a day, nearly all the MAHs and light alkanes would have been lost, mainly by evaporation, and any remaining toxicity to water column organisms would be caused primarily by dissolved PAHs, phenols, and polar components of the oils.

Two recent studies reported adverse toxic effects to pink salmon and herring embryos/larvae during chronic exposure in the laboratory to water-soluble extracts of weathered Alaskan North Slope crude oil containing 0.001 mg/L or less of total PAHs [31,32]. The exposure media were prepared by passing seawater through a column containing weathered crude oil adsorbed to gravel and probably produced an effluent re-

sembling a WAF. As shown here and in other publications [24,25], PAHs usually contribute less than 50% to the toxicity of WAFs of weathered crude oils. The effluents containing 0.001 mg/L total PAHs undoubtedly contained other petroleum components, particularly polar compounds produced by oil degradation [33], that contributed to or were the major cause of the toxic effects observed.

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APPENDIX

Target analytes in fresh and weathered oils and water-accommodated fractions (WAFs); alkylphenanthrenes may include alkylanthracenes and alkylphenols may include benzoic acids^a

<u>GC/MS target PAHs</u>	<u>GC/FID target alkanes</u>	<u>GC/MS target phenols</u>
Naphthalene	<i>n</i> -C ₈ through <i>n</i> -C ₃₆	Phenol
C ₁ -naphthalenes	Pristane	C ₁ -alkylphenols
C ₂ -naphthalenes	Phytane	C ₂ -alkylphenols
C ₃ -naphthalenes	Isoprenoid 1380	C ₃ -alkylphenols
C ₄ -naphthalenes	Isoprenoid 1470	C ₄ -alkylphenols
Biphenyl	Isoprenoid 1650	C ₅ -alkylphenols
Acenaphthylene	TPH	C ₆ -alkylphenols
Dibenzofuran		C ₉ -alkylphenols
Acenaphthene		C ₈ -alkylphenols
Fluorene		C ₇ -alkylphenols
C ₁ -uorenes	<u>GC/MS target MAHs</u>	2- <i>tert</i> -Butyl-4-methylphenol
C ₂ -uorenes	Benzene	2,6-Di-isopropylphenol
C ₃ -uorenes	Toluene	6- <i>tert</i> -Butyl-2,4-dimethylphenol
Anthracene	Ethylbenzene	<i>p</i> - <i>tert</i> -pentylphenol
Phenanthrene	<i>m</i> -Xylene	2,6-Di- <i>tert</i> -butyl-4-methylphenol
C ₁ -phenanthrenes	<i>p</i> -Xylene	2,4-Di- <i>tert</i> -butylphenol
C ₂ -phenanthrenes	<i>o</i> -Xylene	2,6-Di-isopropyl-4-methylphenol
C ₃ -phenanthrenes	Isopropylbenzene	
C ₄ -phenanthrenes	<i>n</i> -Propylbenzene	
Dibenzothiophene	1-Methyl-3-ethylbenzene	<u>Spiking compounds and reporting limits</u>
C ₁ -dibenzothiophenes	1-Methyl-4-ethylbenzene	<u>GC/MS SIS compounds</u>
C ₂ -dibenzothiophenes	1-Methyl-2-ethylbenzene	Naphthalene-d ₈
C ₃ -dibenzothiophenes	1,3,5-Trimethylbenzene	Fluorene-d ₁₀
Fluoranthene	1,2,4/1,2,3-Trimethylbenzene	Chrysene-d ₁₂
Pyrene	<i>sec</i> -Butylbenzene	
C ₁ -uoranthene/pyrenes	1-Methyl-3-isopropylbenzene	<u>GC/FID SIS compound</u>
C ₂ -uoranthene/pyrenes	1-Methyl-4-isopropylbenzene	<i>o</i> -Terphenyl
C ₃ -uoranthene/pyrenes	1-Methyl-2-isopropylbenzene	
Benz[<i>a</i>]anthracene	1-Methyl-3- <i>n</i> -propylbenzene	<u>GC/MS RIS compounds</u>
Chrysene	1-Methyl-4- <i>n</i> -propylbenzene	Acenaphthene-d ₁₀
C ₁ -chrysenes	1,3-Dimethyl-5-ethylbenzene	Phenanthrene-d ₁₀
C ₂ -chrysenes	1,2-Diethylbenzene	Benzo[<i>a</i>]pyrene-d ₁₂
C ₃ -chrysenes	1,3-Diethylbenzene	
C ₄ -chrysenes	1,4-Diethylbenzene	<u>Reporting limits</u>
Benzo[<i>b</i>]uoranthene	1-Methyl-2- <i>n</i> -propylbenzene	<u>Oil/tar</u>
Benzo[<i>k</i>]uoranthene	1,4-Dimethyl-2-ethylbenzene	PAH: 0.2 mg/kg
Benzo[<i>e</i>]pyrene	1,2-Dimethyl-4-ethylbenzene	Alkanes: 100 mg/kg
Benzo[<i>a</i>]pyrene	1,3-Dimethyl-2-ethylbenzene	TPH: 100,000 mg/kg
Perylene	1,3-Dimethyl-4-ethylbenzene	MAH: 100 mg/kg
Indeno[1,2,3- <i>c,d</i>]pyrene	1,2-Dimethyl-3-ethylbenzene	<u>Water</u>
Dibenz[<i>a,h</i>]anthracene	1,2,4,5-Tetramethylbenzene	PAH: 1 ng/L
Benzo[<i>g,h,i</i>]perylene	1,2,3,5-Tetramethylbenzene	MAH: 0.2 mg/L
	1,2,3,4-Tetramethylbenzene	

^a GC/MS 5 gas chromatography/mass spectrometry; GC/FID 5 gas chromatography/ame ionization detection; PAH 5 polycyclic aromatic hydrocarbons; TPH 5 total petroleum hydrocarbons; MAH 5 monocyclic aromatic hydrocarbons; SIS 5 surrogate internal standard; RIS 5 recovery internal standard.